

H-86-3

SOUTHWEST FISHERIES CENTER

HONOLULU, HI 96812

P.O. BOX 3830

February 1986

HONOLULU LABORATORY

NATIONAL MARINE FISHERIES SERVICE

EFFECTS OF COLD SHOCK ON LARVAL AND JUVENILE TROPICAL FISHES: POTENTIAL IMPACTS OF OCEAN THERMAL ENERGY CONVERSION

Yara Lamadrid-Rose and George W. Boehlert
Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96812

This report is used to insure prompt dissemination of preliminary results, interim reports, and special studies to the scientific community. Contact the authors if you wish to cite or reproduce this material.

Southwest Fisheries Center Administrative Report H-86-3

EFFECTS OF COLD SHOCK ON LARVAL AND JUVENILE TROPICAL FISHES:

POTENTIAL IMPACTS OF OCEAN THERMAL ENERGY CONVERSION

Yara Lamadrid-Rose and George W. Boehlert
Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96812

FEBRUARY 1986

NOT FOR PUBLICATION

ABSTRACT

Ocean Thermal Energy Conversion (OTEC) facilities will bring large volumes of deep, cold water to the surface, where it will be mixed with warm surface water after the utilization of the thermal differential. Mixing of these two water types will result in exposure of larval and juvenile fishes entrained in the warmwater intake to lowered temperatures. A time course of cold shock from simulated entrainment through the planned Kahe Point OTEC plant was used to determine potential effects upon larval and juvenile tropical fishes. Experiments were designed to allow testing of different exposure times (8, 16, and 24 min) and temperature differentials (ΔT , 5, 10, and 15 C°) upon different early life stages of tropical fishes.

Eggs and yolk sac larvae were tested for mahimahi, Coryphaena hippurus, and manini, Acanthurus triostegus. Generally, earlier stage eggs were more sensitive to temperature shock, with mortality increasing with increasing ΔT as compared to controls; exposure time, however, did not exert a significant effect. Significant excess mortality for all stages of eggs occurred at the range of times and temperatures proposed in current OTEC engineering plans. For mahimahi larvae, excess mortality increased with age to values higher than for latest stage eggs, but we consider this to be caused by stress associated with yolk exhaustion.

Juvenile striped mullet, Mugil cephalus, were also tested. A single size range was exposed to the same experimental protocol as the eggs and larvae. Although exposure time was not significant, ΔT had a significant effect upon mortality, and there was a significant interaction between exposure time and ΔT .

Sublethal effects of thermal shock were determined by considering the effects of "predation" upon yolk sac larvae of mahimahi. Newly hatched and day 1 posthatch larvae were exposed to three ΔT 's for three exposure periods, at two levels of simulated predation exposure with and without a 1-h recovery period. Generally, excess mortality was greater without a recovery period, for the earlier stage, at higher ΔT , and in the greater "predation" pressure. This suggests that entrained larvae might suffer sublethal effects as well as the direct thermal effects of mortality.

INTRODUCTION

Studies on man-induced thermal effects on aquatic ecosystems have generally considered elevated temperatures resulting from once-through cooling systems of fossil fuel or nuclear powerplants (Van Winkle 1977; Schubel et al. 1978; Talmage and Coutant 1979). The majority of these studies have concentrated on higher latitude areas and freshwater ecosystems, although recent studies have begun to consider marine ecosystems (Hoss et al. 1974; Burton et al. 1976; Smith et al. 1979; Barker et al. 1981). As the utilization of marine waters for cooling increased, concerns about negative impacts upon fisheries have developed (Saila 1975). Although some studies have dealt with thermal effects in marine systems, they have concentrated upon elevated temperatures. With the proposed development of ocean thermal energy conversion (OTEC), however, new concerns have been expressed about exposure to decreased temperatures in the effluent plume. This study considers the effects of lowered temperatures upon tropical fishes, an area not considered in past studies.

A great deal of interest has centered upon effects of thermal stress on fishes; in particular, the egg and larval stages have received considerable attention due to their vulnerability to entrainment and their high sensitivity to thermal stress (Horst 1975; Schubel 1975; Rosenthal and Alderdice 1976). Again, most of these studies have concentrated upon the effects of elevated temperature, primarily as it affects mortality (Smith et al. 1979; Barker et al. 1981). Relatively little work has been conducted upon the lower thermal tolerances of fish larvae or upon the effects of lower temperatures upon development, particularly in tropical systems. Even slightly lowered temperatures, however, may have subtle but important effects upon larval fishes. Lasker (1964), for example, noted normal development of larval Pacific sardine, Sardinops caerulea, at temperatures of 14°-21°C, but no development of a functional jaw at temperatures of 13°C and below. Larvae were unable to initiate feeding at temperatures which were only slightly lower than those observed in nature. Alderdice and Forrester (1971) found that Pacific cod, Gadus macrocephalus, eggs were able to tolerate variability in salinity and dissolved oxygen if within a temperature range of 3°-5°C. Below 3°C, however, egg viability decreased sharply, irrespective of other environmental conditions. Alderdice and Velsen (1971) observed abnormal jaw development in the larvae of the Pacific herring, Clupea pallasii, at temperatures of 4.0°-4.71°C; optimal temperature for viable hatch was 6.85°C at 16.41‰. Hayes et al. (1953) observed that lowered temperatures affected the order of appearance of developmental features in embryos of the salmon, Salmo salar. Lowered temperatures also resulted in abnormal hatching; the larvae typically emerged head or yolk sac first rather than tail first and suffered higher than normal mortalities. May (1974) observed that Bairdiella icistia eggs reared at 18°C failed to develop past the blastula stage despite normal embryonic development between 20° and 30°C. These and other studies demonstrated that temperatures only a few degrees below ambient may cause mortalities in marine fish larvae.

Temperature shocks in nature may also be characterized by sublethal or stress effects, where the probability of subsequent mortality is increased

(Rosenthal and Alderdice 1976; Mazeaud et al. 1977). Sublethal stresses may decrease predator avoidance capabilities in nature (Coutant 1973; Farr 1977). Coutant et al. (1974) observed increased predation in juvenile channel catfish, Ictalurus punctatus and largemouth bass, Micropterus salmoides, that were exposed to varying cold thermal shocks. The incidence of predation increased with increasing difference between normal and shock temperatures.

In the present study we describe the effects of lowered temperatures on egg and larval stages of two representative species of tropical marine fishes, the mahimahi, Coryphaena hippurus, and the manini, Acanthurus triostegus. We also determine the effects upon juvenile striped mullet, Mugil cephalus. By considering pelagic, reef-dwelling, and nearshore species, the results of these experiments should provide valuable data for understanding potential effects of OTEC on tropical marine ecosystems and the relevant fishery resources.

MATERIALS AND METHODS

Experiments were conducted at the Kewalo Research Facility of the Southwest Fisheries Center Honolulu Laboratory, where the capability to naturally spawn pelagic and reef fishes provided experimental animals. The primary species used were the mahimahi, C. hippurus, and the manini, A. triostegus. Although both species have pelagic eggs and larvae, adults of the mahimahi remain pelagic and juvenile manini settle on the reef. Juvenile striped mullet, M. cephalus, were also used in thermal tolerance experiments due to their availability in the shallow waters of Oahu.

Two types of experiments were conducted. The first experiments considered mortality of eggs, larvae, and juvenile fish caused by lowered temperatures. Our experimental design considered an analysis of the time-temperature histories for seawater pumped through and discharged from typical OTEC operations (Myers¹). This design (Fig. 1) is patterned after the engineering plans for the Ocean Thermal Corporation's (1983) planned Kahe Point facility. Warm surface water (74.54 m³/sec at 25.5°C) is drawn into the plant and immediately mixed with the warmwater effluent from the existing oil-fired plant (31.85 m³/sec at 31.3°C), raising the temperature of the mixed water to 27.2°C. As this mixed water flows through the heat exchangers, a small temperature drop results and then the final temperature drops to approximately 16.7°C. The time frame for a change from ambient to mixed temperatures is <1 min. The transit through the mixed effluent pipes would be approximately 8 min (Fig. 1) before release near a depth of 100 m, where a dilution period of about 1 min would bring the temperature back to within 2°C of the surface temperature.

Attempts were made to simulate representative thermal histories in the experiments; an exception is that we did not include the small temperature

¹E. Myers, Ocean Minerals and Energy, NOAA, Washington, D.C. 20235, pers. commun.

increase (Fig. 1) associated with the heated effluent addition before thermal shock. This would have made our design more complex and may have made interpretation difficult. Similar larval fish experiments have been conducted by Smith et al. (1979), who used a square wave exposure design; larvae acclimated to normal temperatures were exposed for varying periods to the alternate temperature and then returned to ambient temperatures. In our design, the experimental variables considered are stage of egg or larval development, magnitude of thermal shock (ΔT), and duration of lowered temperature exposure. Developmental stage classification for mahimahi was obtained from Palko et al. (1982); manini eggs were staged using Oppenheimer's (1937) classifications. The values for ΔT and exposure times were chosen to encompass those of the proposed design. Since the proposed ΔT is approximately 8°C (Fig. 1), values chosen for our work were 5 , 10 , and 15°C . Exposure times of 8 , 16 , and 24 min were chosen for values for our experiments.

The second series of experiments considered the sublethal effects of lowered temperatures on larval mahimahi. In these experiments, two stages of yolk sac larvae were used. Larvae were exposed to a temperature shock varying in time and magnitude, as above, and then returned to a tank with a predator. Various planktivorous fishes found in Hawaiian waters were tested as potential predators. None of the species tested were able to successfully adapt to the experimental tanks. Instead, exposure to a constant flow siphon system was used to simulate a predator.

Thermal Exposure Experiments

Coryphaena hippurus, Mahimahi

Mahimahi brood stocks were kept at the Kewalo Research Facility in various flow-through circular tanks at ambient temperatures of 23.5 to 24.5°C ; tanks of two different sizes (5 or 7.2 m diameter), depending on availability, were used to hold fish.

During the course of the experiments, the brood stocks spawned every other day without artificial induction. Mahimahi eggs are spherical (1.52 – 1.66 mm in diameter), buoyant, and have a single oil globule (0.31 – 0.34 mm in diameter). At an ambient water temperature of 26°C , the eggs hatched approximately 54 h after spawning; the larvae ranged from 4.3 to 5.4 mm standard length (SL).

The experimental design was as follows: A complete series of experiments was composed of three tests; each test consisted of one ΔT with samples from six developmental stages at three exposure times including a control and replicates. The developmental stages used were three egg stages (0 , 12 , and 24 h postfertilization) and three larval stages (day of hatch, day 2 , and day 4 posthatch). Three exposure times (8 , 16 , and 24 min), as well as a control were used for each of three temperature shocks (ΔT , 5 , 10 , and 15°C) below the ambient temperature (26°C). Three replicate samples were used for each of the 3 exposure times and the control; thus there were 12 samples for a single developmental stage per temperature drop and a total of 72 samples per test. Fifty eggs or 20

larvae per replicate were used in these experiments. For each test, the eggs and larvae used were obtained from a single spawning day. Specific controls for each spawning were necessary because egg quality may vary among spawnings (Kazama²).

Mahimahi eggs were collected within 10-20 min after spawning. The eggs were gathered in a fine mesh dip net as they floated along the surface of the water; they were placed in a 22.8-liter (6-gallon) bucket with seawater from the parental tank. The procedure was repeated until 4,000-5,000 eggs, a number sufficient to conduct a test, were collected. The eggs were immediately taken into the laboratory and mildly aerated. Samples were removed and checked for normal fertilization and development. They were then placed in a 100-liter tank within a water bath tank maintained at 26°C. The 100-liter tank and the water bath contained aerated seawater filtered through a 0.22 μ m Millipore³ filter system. A photo period with 18 h of light and 6 h of darkness (18L:6D) was maintained with timers on a fluorescent light system directly above the water bath tank. Marine microalgae (Tetraselmas sp. and Isochrysis sp.) were added in small quantities to the 100-liter tank and the ambient water bath tank to maintain water quality. On day 1 post-hatch, the 100-liter tank was inoculated with rotifers (Brachionus sp.) at the concentration of 1-2/ml. This food density was maintained until the termination of the experiments at day 5 posthatch. At 26°C yolk was absorbed by day 2 posthatch.

Egg and larval samples were placed in 1-liter plastic beakers modified for water transfer by cutting six windows symmetrically along the top of the beaker. The windows were covered with 350 μ m Nitex mesh attached with aquarium grade silicone caulking material which was cured and soaked in freshwater to leach out potential toxins. Beakers were labeled and the eggs or larvae randomly distributed among the beakers. The beakers were suspended in the ambient temperature (26°C) water bath by rings of styro-foam. A fine-pore airstone was suspended in each beaker and the seawater gently aerated. The beakers to be subjected to the thermal shock were individually transferred from the ambient temperature water bath tank (holding tank) to the treatment tank (shock tank). The shock tank was maintained at the desired temperature for the test--delta T of 5 (21°C), 10 (16°C), or 15 C° (11°C, all $\pm$ 0.5°C)--by use of a cooling unit in the shock tank.

The transfer was made by allowing the ambient seawater in the beaker to drain to the bottom of the screened window, approximately one-fourth the height of the beaker. It was then placed in the shock tank and the colder seawater allowed to bring the water volume to 1 liter. Then 4 liters of seawater from the shock tank were siphoned into each 1-liter test beaker

²T. Kazama, Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, NOAA, Honolulu, HI 96812, pers. commun.

³Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

through a 5-mm inside diameter (ID) hose. This produced a 1-min temperature drop from the ambient temperature (26°C) to the desired shock temperature. This procedure was repeated for each of nine test beakers per stage exposed to the thermal shock. The three control beakers per stage were handled identically except that instead of transferring the control beakers to the shock tank, the control beakers were placed back into the holding tank and an equal volume of ambient seawater was used in the exchange.

After the appropriate exposure time in the shock tank, each test beaker was again allowed to drain to the bottom of the screened window, returned to the holding tank, and, over a period of 1 min, flooded with ambient seawater from the water bath to bring the temperature up to 24°C. The beaker was left in the holding tank so that the water temperature could slowly return to the ambient (26°C); this required approximately 1 h. The test beakers were kept in the holding tank at ambient temperature until the termination of the test. The control beakers were handled in the same manner but with no thermal shock.

The egg samples were held in the test beakers until day 1 posthatch. Mortality and development data were recorded daily. Approximately 12 h after the thermal shock occurred, the egg samples exposed to the shock and the control samples were checked for mortalities and incidences of abnormalities as described by Rosenthal and Alderdice (1976). Thereafter, eggs were checked every 24 h until day 1 posthatch recording of mortality or abnormality. On day 1 posthatch, the egg samples were terminated; surviving and dead eggs were counted and recorded. Observations of abnormalities in the eggs and larvae, as well as time delays in hatching, were also recorded. Survivors were not used in subsequent experiments.

Methodology for experiments with larvae was similar. Dead larvae were removed 1 h after the thermal shock, and then kept in the test beakers for periods ranging between 16 and 24 h postshock. When the experiment was terminated, the number of dead and surviving was recorded. Survivors were not used in subsequent experiments.

Egg and larval samples were labeled and preserved in 3% Formalin (in seawater) during two tests conducted at ΔT 5°C. Dead eggs found at observation intervals were recorded, labeled, and preserved; at the termination of the experiment, surviving and dead eggs were preserved. Dead larvae found 1-h postshock were labeled and preserved; when the experiment was terminated, surviving and dead larvae were counted and preserved.

Acanthurus triostegus, Manini

Manini brood stocks were kept at the Kewalo Research Facility in a 7.2-m diameter tank at ambient temperatures of 23.1° to 23.4°C. Various species of tuna were usually also in the tank but did not interfere with the spawning activity of the manini. During the experiments, the brood stock spawned naturally two to three times a week until mid-October 1984, after which spawning became less frequent (once a week at most). A few days before periods of a full moon, however, spawning activity increased. Manini eggs are spherical, 0.63-0.70 mm in diameter, buoyant, and have a

single oil globule (0.15–0.18 mm in diameter). At an ambient seawater temperature of 26°C, the eggs hatched approximately 36 h after spawning. At hatching, the larvae ranged from 2.03 to 2.43 mm in length.

The experimental design was identical to the mahimahi procedures with some exceptions. Only three developmental stages were used for each test (two egg stages, 0 and 18 h postfertilization, and one yolk sac larval stage, day 1 posthatch). The complete series of experiments consisted of three tests, each with one delta T, three developmental stages at three exposure times with a control, and three replicates of each. The exposure times and the temperature drops were the same. A test, therefore, consisted of three 12-sample beaker runs at each temperature drop. As in the previous experiments, 50 eggs and 20 larvae were used per replicate.

Manini eggs were collected within 10–30 min after spawning. The eggs were handled and processed in the same manner as the mahimahi eggs. The beakers used in these experiments were similar to those used for mahimahi except that the bottom was painted with black epoxy resin paint to facilitate observations of the small, semitransparent larvae. Also, the windows were covered with 250 µm Nitex mesh. The beakers were soaked to leach out potential toxins. Individual beakers were not aerated in these experiments, but the water bath was vigorously aerated. The test beakers were transferred and exposed to the thermal shocks as in the mahimahi experiments. The only microalga used as water conditioner in these experiments was Isochrysis sp.

The procedure by which egg and larval samples were treated after the thermal shocks differed from previous experiments. The egg samples were held in the test beakers until day of hatch. Due to the small size and fragile nature of the larvae, mortality and abnormality data were recorded only at the termination of each experiment. Mortality, survival, and abnormality of eggs and larvae, as well as time delays in hatching, were also recorded at this time. Survivors were not used in subsequent experiments. Larval samples were held for 24 h postshock; the numbers of deaths and survivors were similarly recorded at the termination of experiments. At the termination of each test all egg and larval samples were labeled and preserved in 3% Formalin (in seawater).

Mugil cephalus, Striped Mullet

Mullet juveniles were collected between 8 November and 18 December at Waialae Beach Park, Honolulu, Hawaii. The juveniles were kept in 2-m circular flow-through tanks in water between 25° and 26°C. They were fed a varied diet including trout and catfish chow, live brine shrimp, Artemia sp., and commercial flake fish food. The fish used in the experiments ranged from 25.8 to 66.5 mm SL.

The basic experimental design was similar to that used with larval stages, except that the juvenile experiments were conducted on a larger scale. A complete series of experiments consisted of three tests. Only one stage (juvenile fish) was used in each test, but exposure times and delta T were the same as those in the other experiments. Two complete series of

experiments were run. In the first series, three replicates were used for each exposure time and control; in the second series, only two replicates were used due to the availability of fish. Ten juveniles were used for each sample.

Juveniles were randomly transferred from the outside holding tanks to 38-liter (10-gallon) glass aquaria in groups of 5 fish per tank until each tank contained 10 fish. A 200-W submersible heater in each tank maintained a 26°C ambient seawater temperature; water was circulated by use of an air-stone suspended in each tank. The alga, Tetraselmas sp., was added in small quantities to maintain water quality. The fish were allowed to acclimate in the tanks for 4 to 5 h before the exposure experiments were conducted. Fish in tanks to be subjected to the thermal shock were transferred to a 4-liter bucket 5 min before the exposure. The buckets were modified to facilitate water exchange; each bucket had 3-mm holes uniformly cut from the top to the 1.5-liter level. The fish were thus transferred to the shock tank in approximately 1.5 liters of ambient seawater. Gradually, the bucket was submerged and flooded with the colder seawater; this produced a 1-min temperature drop from the ambient temperature to the desired shock temperature ($\pm 0.5^\circ\text{C}$). The process was repeated for each of the test tanks. The control tanks were handled identically except that they were placed back into their respective holding tank and submerged and flooded with ambient seawater.

Two minutes before termination of the exposure, colder seawater was added to the holding tanks to reduce the temperature to 24°C. After the appropriate exposure time for each sample, buckets were drained to the 1.5-liter mark and gradually submerged and flooded with seawater in the holding tank. This brought the test fish to 24°C in 1 min. The heaters raised the temperature to 26°C over a period of 30 min to 1 h. The control fish were handled in the same method, except that the water was maintained at the ambient temperature (26°C). The fish were kept in the holding tanks for 24 to 48 h postshock.

In the first series of experiments with three replicates, only dead fish were labeled and preserved; also, two fish from each holding tank were measured before they were released. In the last test in the series ($\Delta T = 5^\circ\text{C}$), fish that had been used in the $\Delta T 10^\circ\text{C}$ were among those used in the exposures. Also, at $\Delta T 5^\circ\text{C}$, two surviving fish from each tank were measured and preserved. Measurements were also made on dead fish; the fish were labeled and preserved in 10% Formalin (in seawater). In the second series of experiments with two replicates, all fish were labeled and preserved at the termination of the test in 10% Formalin (in seawater).

Predation Experiments

Mahimahi Larvae

Mahimahi eggs were collected within 6-8 h after spawning, placed in 100-liter tanks, and allowed to develop as previously described for the mahimahi thermal shock experiments. Yolk sac larvae were exposed to cold thermal shocks as described for previous experiments. Day of hatch and day

1 yolk sac larvae were the two stages used. Two types of predation experiments were conducted. The first experiments tested the ability of the larvae to avoid a predator immediately after exposure to thermal shock; the second tested this avoidance ability after a recovery period of 1 h. Although some fishes were tested as potential predators in the experiments, it was difficult to develop uniform feeding motivation; thus behavioral factors associated with the predator would have been an additional factor which would have added variability to the results. We therefore tested a stochastic "simulated predator" which consisted of a 5-mm siphon tube placed at a consistent level about 4 cm deep in the beaker. The predator tank system was set up in the following manner. A central reservoir tank was fed a constant supply of filtered seawater; this tank in turn supplied a constant water flow to a 5-liter beaker by means of a 5-mm inner diameter input siphon. This beaker was the holding beaker; the "predator siphon" was fixed into position on the holding beaker so that the water output would be constant and from the same site. The predator siphon drained into a 1-liter "recovery" beaker lined with Nitex mesh screening and suspended in the water column with rings of styrofoam. This system produced a constant flow rate which resulted in the predator effect of larvae being siphoned out of the holding beaker. A duplicate system of 6 holding beakers was set up so that a complete experiment of 12 samples could be conducted at one time. The output flow rate of the predator siphon was set at two levels, from 130 to 165 ml/min and 80 to 120 ml/min. The flow rates were set daily at the beginning of each experiment and rechecked periodically during the experiment. After the larvae were placed in the holding beakers, the experiments were run for 2 h at the 130-165 ml/min flow rate, and 3 h at the 80-120 ml/min flow rate. The water in the predator tank system, because of the large volume of seawater used, was run at ambient seawater conditions, i.e., 23.6° to 24.2°C.

In the first experiments, the larvae were placed into the holding beakers of the predator tank system immediately after removal from the cold shock tank. When the larvae were put in the holding beaker, the output siphon was covered for 30 sec to prevent larvae from being removed due to initial turbulence. Larva deaths were counted in the 1-liter recovery beakers at 10-min, 1-h, and 2-h intervals at the 130-165 ml/min flow rates and at 10-min, and 1-, 2-, and 3-h intervals at the 80-120 ml/min flow rates. The experiment was then terminated and the numbers of survivors and deaths in the 5-liter holding beaker, as well as the number in the recovery beaker, were recorded. Survivors were not used in subsequent experiments. In the second type of experiments, a 1-h recovery period was introduced between removal of the larvae from the cold shock tank and placing them into the predator tank system. The remainder of the predation experiment was conducted as described above.

Data analysis consisted primarily of three-way analysis of variance (ANOVA) with Duncan's multiple range (Nie et al. 1975) test to compare treatment means. Two ANOVA's were run for each experiment. The data analyzed in the first were proportion survival, with factors delta T (three levels), stage of development (varying levels), and exposure time (four levels, where controls comprised zero exposure time). In the second ANOVA, the datum analyzed was proportion excess mortality. Since control mortality

varied with egg quality, mean mortality values for controls were subtracted from the relevant treatment values to determine "excess mortality." Both values, as proportions were arcsin transformed before running the ANOVA.

RESULTS

Thermal Exposure Experiments

Coryphaena hippurus, Mahimahi

Five complete tests were conducted using six stages of mahimahi eggs and larvae with three tests at 5 C°, one test at 10 C°, and one test at 15 C°. For 0 h postfertilization eggs, drops of 10 and 15 C° produced 100% mortalities within 12 h of exposure to the cold shock (Fig. 2; Table 1). These eggs, shocked between the 2 and 16 cell stage, failed to develop beyond the gastrula stage and died. The control eggs, from the same age group, however, were developing normally and at the early neurula stage. Newly fertilized eggs exposed to 5 C° delta T had higher excess mortalities than later stages exposed to the same delta T and exposure time (Table 2). The 12 h postfertilization eggs were shocked at the late gastrula-early neurula stage. Again, the greatest excess mortalities were observed at delta T 15 C°, and decreased at the lower delta T's (Table 2). The 24 h postfertilization eggs were exposed to thermal shock at the developing embryo stage: The embryo occupied approximately one-half the circumference of the egg, the optic vesicles were formed, and the yolk was pigmented. Excess mortality of the eggs exposed at this stage was significantly lower than eggs exposed at earlier stages. Excess mortality increased in eggs exposed to greater delta T's, but overall excess mortality in this age group was relatively low.

In all the egg stages shocked and reared to hatching in the 1-liter beakers, deaths were observed on the day of hatch (Table 3). This was also true for the controls in the 1-liter beakers. This increased mortality on day of hatch, however, was not observed in the 100-liter rearing tank. Perhaps the small size of the 1-liter beakers induced mechanical damage which resulted in these mortalities, but excess mortality over controls was clearly seen (Table 4). One thing which should be pointed out for these experiments concerns mortalities of the controls. Typically, the control mortalities are highest for the earliest stage eggs (Tables 1-4). In our experiments, only live eggs were used in the start of the experiments. Thus even in the holding tanks, egg mortalities may have occurred before eggs were used in later-stage egg experiments. Early stages of development are likely to have the highest incidence of natural mortalities due to deformations or genetic defects (Vladimirov 1975; Longwell and Hughes 1981). This is indeed observed in the percentages of abnormalities in the controls; the highest incidence of abnormalities was in the newly fertilized eggs (Table 5).

Abnormalities observed in these experiments included yolk and oil globule deformities, bent body, tail and head deformities, and an amorphous embryo mass. Abnormalities in 0 h postfertilization eggs were more common than in eggs exposed to cold thermal shock at later stages (Table 5); the

incidence of abnormalities in the 12 h postfertilization eggs was greater than that in eggs shocked at 24 h postfertilization. The 12 and 24 h stage egg data could not effectively be compared to the 0 h stage egg data due to the 100% mortalities at the higher delta T's (Table 5). The percentage of abnormalities in the 24 h postfertilization, however, was very low.

The results for the larval stages were almost inverse that seen for the egg stages. The newly hatched larvae have lower excess mortalities than day 2 and day 4 posthatch feeding larvae. Neither the delta T nor the exposure significantly affects the survival of the day of hatch larvae (Table 6). The day 2 and day 4 larvae have significantly higher excess mortality than day of hatch larvae (Table 7). In our experiments, all larvae showed similar reactions to the cold shock; they turned slightly dark, sank to the bottom of the beaker, and were less active. When returned to the ambient temperature water bath, they quickly resumed their normal coloration and position in the water column. The degree and speed of their response to the cold thermal shock were directly proportional to the magnitude of delta T. The greater mortalities seen in day 2 and day 4 larvae may have been a result of starvation. Although, the larvae were fed before the experiments, the rapid metabolism of these larvae at 26°C could have resulted in sublethal stress within the 24 h postshock period; the excess mortalities (Table 7) may have been the result of the additional stress of the thermal shock.

Analyses of the mahimahi egg and larva data, including the treatment of eggs and larvae separately and combined, are presented in Table 8. Interaction effects of the factors are not considered. Generally, the ANOVA analysis on the excess mortality most clearly demonstrates the important effects; these data are presented graphically in Figure 3. Overall, the most significant effects were from delta T and developmental stage, and exposure time had no effect (the effect of exposure time in the analysis of percent mean mortality is significant, but this is due to the inclusion of controls as zero exposure time; this group is significantly less in all cases but treatment means are equal). The effects of delta T were apparent for all data combined and for eggs, but not for larvae alone. For the combined analysis, the 5 C° differed from the 10 and 15 C° data, whereas for the eggs, the treatment means increased with increasing delta T. As a function of developmental stage, in the combined analysis, excess mortality for newly fertilized eggs is significantly greater than that for the group with 12 h eggs and all three larval groups; the 24 h postfertilization eggs suffer significantly lower excess mortality than either group. For eggs alone, each group differs in the same manner as in the combined analysis, whereas the larval groups analyzed alone are not significantly different.

Acanthurus triostegus, Manini

Three complete tests, one at each delta T, were conducted using the three stages of manini eggs and larvae (Table 9). The results (Fig. 4) were similar to those in the mahimahi experiments. For newly hatched eggs, mortalities increased with increasing delta T (Table 9). The later egg stage (18 h postfertilization) had greatly decreased mortalities at all delta T's. Because intermittent observations were not made on the larvae, and because the hatched larvae were very fragile, abnormality data were not

obtained. The day 1 yolk sac larvae, including the controls showed very high mortalities (Table 9; Fig. 4); when calculated as excess mortality (Table 10), no trends related to delta T's or exposure period were apparent. The manini data showed significant effects of all variables when mean percent mortality data were included, but exposure time was not significant when considering excess mortality (Table 11). Percent excess mortality for 5 and 10 C° delta T was not significantly different, but both differed from 15 C° delta T. The only significant effect of developmental stage was that 18 h postfertilization eggs had a very low mortality (Table 11).

Mugil cephalus, Mullet

The juvenile mullet were used in two complete series of experiments. In the first series, larger fish (mean 50.1 mm SL) were used in three tests at each delta T with three replicates. In the second series, smaller fish (mean 33.0 mm SL) were used with two replicates. The size difference between the groups of fish was significant (t-test, $P < 0.01$), but there was no significant difference in excess mortality (t-test, $P > 0.05$; Table 12); data from both series of experiments were thus combined. The juvenile mullet showed no significant excess mortality at 5 and 10 C° delta T (Fig. 5). Behaviorally, juveniles placed into the cold shock at that temperature typically turned dark, decreased activity, and remained near the bottom of the container. A few of the fish at 10 C° delta T swam in quick, jerking motions for a few seconds before settling like the others. At 15 C° delta T, however, the fish placed in the water swam in similar quick, and jerking motions with their opercula flared and lost equilibrium after a few seconds. Most of the fish that exhibited this behavior did not recover and died; 25% of the fish did recover and survived to the end of the experiment (Table 12). In these experiments, unlike the ones above, exposure time did have a significant effect on excess mortality within the 15 C° delta T. As the exposure time increased, excess mortality also increased (Tables 12, 13).

Predation Experiments

Mahimahi Larvae

Two complete series of experiments were conducted using two stages of mahimahi yolk sac larvae. Each series was run with and without a 1-h recovery period between the thermal shock and transferring the larvae into the predator tank. The first series was run at a faster flow rate (130-165 ml/min) than the second series (80-120 ml/min); the difference between results for the two flow rates was not marked, but excess mortality for the faster flow rate was greater for a recovery period (cf. Table 14 with 15). Excess mortality increased with increasing delta T, as well as with increasing exposure time (Table 16). There was also a significant difference between the two larval stages and their reaction to predation. Newly hatched larvae were very buoyant and floated parallel to and on the water surface. Day 1 larvae were less buoyant and remained either in midwater or on the bottom; their heads were typically oriented toward the bottom. Gentle aeration in these experiments helped alleviate this difference. The ability of day 1 larvae to detect and escape the simulated predator was noticeably greater than the detection and escape response of newly hatched

larvae. These data show several trends. As mentioned above, the difference between the two experiments, which used different flow rates for the simulated predator, occurred when a recovery period was included. The faster flow rate of experiment I typically resulted in greater excess mortality if a 1-h recovery period was used. Within experiments there is generally a greater excess mortality with no recovery period as compared to a 1-h recovery period in the absence of the simulated predation (Table 17). Considering experiment I, without a recovery period, there were no significant effects of either delta T or developmental stage, although all treatments had significant excess mortality. Exposure time showed an unexpected effect; the shortest exposure time was characterized by the greatest excess mortality (this was not observed in experiment II). With a recovery period, all three variables showed significant differences among treatments (Table 17). The greatest excess mortality was apparent at the highest delta T, the later developmental stage, and the shortest exposure time (again not apparent in experiment II).

DISCUSSION

Survival of fishes through early life history stages is an important aspect of population dynamics and should be studied when adverse environmental impacts may affect it. Oviparous fishes which produce large numbers of small eggs obviously suffer high mortality in early life history stages. The major sources of mortality for these early stage larvae are starvation and predation (Hunter 1976, 1981). Environmental perturbation or pollution may clearly increase mortalities during these early life history stages through either direct effects (Hunter et al. 1979; Longwell and Hughes 1981) or sublethal effects (Rosenthal and Alderdice 1976). Thermal pollution, depending upon the magnitude, may be either lethal or sublethal to aquatic organisms.

High temperatures, such as those encountered in typical nuclear or oil-fired electrical generation operations, have been shown to have potentially negative impacts upon early life-history stages of marine fishes (Smith et al. 1979; Talmage and Coutant 1979; Barker et al. 1981). These studies, however, have typically been conducted in temperate regions, where fishes may be more eurythermal than in tropical regions. Fish kills from either cold weather or intrusions of cold water have been documented in tropical regions (Bohnsack 1983), demonstrating that tropical fishes generally have less tolerance to low temperature shock than temperate fishes; further, acclimation to low temperature is typically slower than to high temperature (Doudoroff 1942). Early life history stages of fishes may also be adapted to the upper range of temperatures experienced by a species, as shown by laboratory studies (Medvick and Miller 1979), distributional studies (Norris 1963), and growth studies (Boehlert 1982; Boehlert and Yoklavich 1983). Thus cold shock may exert a strong negative effect upon early life stages of tropical fishes.

Our results suggest that lowered temperatures likely to be encountered by a fish egg or larva in an OTEC operation (Fig. 1) may indeed cause increased mortalities. The highest excess mortalities due strictly to the thermal effects occur for the earliest developmental stages of eggs for

mahimahi (Figs. 2-3; Tables 2, 4, 7) and manini (Fig. 4; Table 10). Whereas later stage eggs suffer less thermal mortality, larvae show particularly high mortality in later stages, but this occurs in the controls as well, implicating either problems with the rearing system or starvation-related effects. Nonetheless, the significant excess mortalities in the larval stages show that sublethal effects may be exerted upon these stages, making the probability of subsequent mortality higher (Mazeaud et al. 1977). For the juvenile fish considered in this study, significant mortality was only observed at ΔT 15 C° (Fig. 5). This is considerably higher than planned temperature changes for a parcel of water entrained in the warmwater intake (Fig. 1).

Entrainment of fish eggs or larvae through an OTEC facility would result in several stresses, including thermal (Fig. 1), pressure and mechanical effects, exposure to biocides, and displacement from the primary habitat. Matsumoto⁴ suggested that these effects would result in 100% mortality of entrained fish eggs and larvae. Our results suggest that the thermal stresses alone will not result in such high mortality, but synergism of thermal effects with the other direct impacts (Hoss et al. 1977) may result in high mortalities before the larvae leave the system in the effluent. Moreover, sublethal effects, including developmental anomalies, deformation, increased vulnerability to predation, and displacement from feeding areas, may occur at this point. For these reasons, and lacking data to the contrary, we support Matsumoto's assumption of 100% mortality of entrained fish eggs and early stage larvae.

LITERATURE CITED

- Alderdice, D. F., and C. R. Forrester.
1971. The effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (Gadus macrocephalus). J. Fish. Res. Board Can. 28:883-901.
- Alderdice, D. F., and F. P. J. Velsen.
1971. Some effects of salinity and temperature on early development of Pacific herring (Clupea pallasii). J. Fish. Res. Board Can. 28:1545-1560.
- Barker, S. L., D. W. Townsend, and J. S. Hacunda.
1981. Mortalities of Atlantic herring, Clupea h. harengus, smooth flounder, Liopsetta putnami and rainbow smelt, Osmerus mordax, larvae exposed to acute thermal shock. Fish. Bull., U.S. 79:198-200.

⁴Matsumoto, W. M. 1984. Potential impact of ocean thermal energy conversion (OTEC) operation on fisheries. Southwest Fish. Cent. Honolulu Lab., Natl. Mar. Fish. Serv., NOAA, Admin. Rep. H-84-4, 29 p.

Boehlert, G. W.

1982. Ontogenetic changes in growth and their relationship with temperature and habitat change. In G. M. Cailliet and C. A. Simenstad (editors), GUTSHOP '81. Fish food habits studies, p. 115-123. Proceedings of the Third Pacific Workshop, December 6-9, 1981, Pacific Grove, California. Wash. Sea Grant Prog., Univ. Wash., Seattle, WSW-WO 82-2.

Boehlert, G. W., and M. M. Yoklavich.

1983. Effects of temperature, ration, and fish size on growth of juvenile black rockfish, Sebastes melanops. Environ. Biol. Fishes 8:17-28.

Bohnsack, J. A.

1983. Resiliency of reef fish communities in the Florida Keys following a January 1977 hypothermal fish kill. Environ. Biol. Fishes 9:41-53.

Burton, D. T., L. B. Richardson, S. L. Margrey, and P. R. Abell.

1976. Effects of low ΔT powerplant temperature on estuarine invertebrates. J. Water Pollut. Control Fed. 48:2259-2272.

Coutant, C. C.

1973. Effects of thermal shock on vulnerability of juvenile salmonids to predation. J. Fish. Res. Board Can. 30:965-973.

Coutant, C. C., H. M. Ducharme, Jr., and J. R. Fisher.

1974. Effects of cold shock on juvenile channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus salmoides) to predation. J. Fish. Res. Board Can. 31:351-354.

Doudoroff, P.

1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with Girella nigricans (Ayres). Biol. Bull. (Woods Hole) 83:219-244.

Farr, J. A.

1977. Impairment of antipredator behavior in Palaemonetes pugio by exposure to sublethal doses of parathion. Trans. Am. Fish. Soc. 106:287-290.

Hayes, F. R., D. Pelluet, and E. Gorham.

1953. Some effects of temperature on the embryonic development of salmon (Salmo salar). Can. J. Zool. 31:42-51.

Horst, T. S.

1975. The assessment of impact due to entrainment of ichthyoplankton. In S. B. Saila (editor), Fisheries and energy production: A symposium, p. 107-118. Heath Publ. Co., Lexington, Mass.

Hoss, D. E., L. C. Clements, and D. R. Colby.

1977. Synergistic effects of exposure to temperature and chlorine on survival of young-of-the-year estuarine fishes. *In* Physiological responses of marine biota to pollutants, p. 345-355. Academic Press, N.Y.

Hoss, D. E., W. F. Hettler, Jr., and L. C. Coston.

1974. Effects of thermal shock on larval estuarine fish--ecological implications with respect to entrainment in power plant cooling system. *In* J. H. S. Blaxter (editor), Early life history of fish, p. 357-371. Springer-Verlag, N.Y.

Hunter, J. R.

1976. Report of a colloquium on larval fish mortality studies and their relation to fishery research, January 1975. U.S. Dep. Commer., NOAA Tech. Rep. NMFS Circ-395, 5 p.

1981. Feeding ecology/predation. *In* R. Lasker (editor), Marine fish larvae: Morphology, ecology and relation to fisheries, p. 34-77. Washington Sea Grant Program, Seattle.

Hunter, J. R., J. H. Taylor, and H. G. Moser.

1979. Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, Engraulis mordax, and Pacific mackerel, Scomber japonicus, during the embryonic stage. Photochem. Photobiol. 27:1-14.

Lasker, R.

1964. An experimental study of the effect of temperature on the incubation time, development, and growth of Pacific sardine embryos and larvae. Copeia 1964:399-405.

Longwell, A. C., and J. B. Hughes.

1981. Cytologic, cytogenetic, and embryonic state of Atlantic mackerel eggs from surface waters of the New York Bight in relation to pollution. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:76-78.

May, R. C.

1974. Effects of temperature and salinity on yolk utilization in Bairdiella icistia (Jordan and Gilbert) (Pisces: Sciaenidae). J. Exp. Mar. Biol. Ecol. 16:213-225.

Mazeaud, M. M., F. Mazeaud, and E. M. Donaldson.

1977. Primary and secondary effects of stress in fish: Some new data with a general review. Trans. Am. Fish. Soc. 106:201-212.

Medvick, P. A., and J. M. Miller.

1979. Behavioral thermoregulation in three Hawaiian reef fishes. Environ. Biol. Fishes 4:23-28.

Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent.

1975. Statistical package for the social sciences, 2d ed. McGraw Hill, N.Y., 675 p.

Norris, K. S.

1963. The functions of temperature in the ecology of the percoid fish Girella nigricans (Ayres). Ecol. Monogr. 33:23-62.

Ocean Thermal Corporation.

1983. Ocean Thermal Energy Conversion (OTEC) site specific environmental assessment. Document No. L3 submitted to Department of Energy under Contract No. DE-AC01-82CE30716. Ocean Thermal Corp., N.Y. (Draft)

Oppenheimer, J. M.

1937. The normal stages of Fundulus heteroclitus. Anat. Rec. 68:1-15.

Palko, B. J., G. L. Beardsley, and W. J. Richards.

1982. Synopsis of the biological data on dolphin-fishes, Coryphaena hippurus Linnaeus and Coryphaena equiselis Linnaeus. U.S. Dep. Commer., NOAA Tech. Rep. NMFS Circ. 443, FAO Fish. Biol. Synop. 130, 28 p.

Rosenthal, H., and D. E. Alderdice.

1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Board Can. 33:2047-2065.

Saila, S. B. (editor).

1975. Fisheries and energy production. Heath Publ. Co., Lexington, Mass., 300 p.

Schubel, J. R.

1975. Some comments on the thermal effects of power plants on fish eggs and larvae. In S. B. Saila (editor), Fisheries and energy production, p. 31-54. Heath Publ. Co. Lexington, Mass.

Schubel, J. R., C. C. Coutant, and P. M. J. Woodhead.

1978. Thermal effects of entrainment. In J. R. Schubel and B.C. Marcy (editors), Power plant entrainment: A biological assessment, p. 19-73. Academic Press, N.Y.

Smith, C. F., J. R. Schubel, M. P. Greges, N. Itzkowitz, S. J. DiPiero, J. Longo, and M. A. Morgan.

1979. Thermal resistance characteristics of early life history stages of finfish from Long Island waters. Mar. Sci. Res. Cent. Spec. Rep. (Stony Brook) 26:79-9.

Talmage, S. S., and C. C. Coutant.

1979. Thermal effects. J. Water Pollut. Control Fed. 51:1517-1554.

Van Winkle, W. (editor).

1977. Assessing the effects of powerplant induced mortalities on fish populations. Pergamon Press, N.Y., 380 p.

Vladimirov, V. I.

1975. Critical periods in the development of fishes. J. Ichthyol.
15:851-868.

Table 1.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, egg stages. Percent mean mortality of three egg stages 12 h postthermal shock exposure data. The delta T 5 C° values are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent mean mortality		
		0 h post- fertilization eggs	12 h post- fertilization eggs	24 h post- fertilization eggs
5	8	31.98	6.65	0.41
	16	25.59	14.52	0.20
	24	26.16	11.21	0.88
	Control	20.23	9.69	0.41
10	8	100.00	7.50	0
	16	100.00	12.71	1.39
	24	100.00	11.67	2.14
	Control	2.27	2.03	0
15	8	100.00	63.99	0.85
	16	100.00	88.90	0
	24	100.00	84.52	8.15
	Control	15.20	7.48	2.67

Table 2.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, egg stages. Percent mean excess mortality of three egg stages 12 h postthermal shock exposure data. Excess mortality was calculated by subtracting the mean of the control from each replicate value, then calculating the final mean from these adjusted values. The delta T 5 C° values are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent excess mortality		
		0 h post- fertilization eggs ¹	12 h post- fertilization eggs	24 h post- fertilization eggs
5	8	18.93	1.33	0.33
	16	14.11	8.79	0.16
	24	14.02	5.80	0.80
10	8	97.73	5.47	0
	16	97.73	10.68	1.39
	24	97.73	9.64	2.14
15	8	84.80	56.51	0
	16	84.80	81.42	0
	24	84.80	77.04	5.48

¹At delta T's 10 and 15 C°, 100% mortality occurred in the test samples at 0 h postfertilization egg stage. The numbers are <100% due to fluctuations in control samples mortalities.

Table 3.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, egg stages. Percent mean abnormality observed in three egg stages throughout development and hatching process. The delta T 5 C° values are means of two separate experiments.

Delta T (C°)	Exposure time (min)	Percent abnormality		
		0 h post- fertilization eggs ¹	12 h post- fertilization eggs	24 h post- fertilization eggs
5	8	4.06	0.96	0
	16	3.18	1.18	0
	24	5.80	1.91	0
	Control	2.44	0.80	0
10	8	--	2.69	0
	16	--	2.82	5.80
	24	--	8.30	0
	Control	1.36	2.13	0
15	8	--	7.43	0
	16	--	0.71	0.72
	24	--	2.73	2.24
	Control	1.37	0.72	0

¹At delta T's 10 and 15 C°, 100% mortality occurred in the test samples at 0 h postfertilization egg stage.

Table 4.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, egg stages. Percent mean mortality of three egg stages on the day of hatch, post-thermal shock exposure data. The delta T 5 C° values are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent mean mortality		
		0 h post- fertilization eggs	12 h post- fertilization eggs	24 h post- fertilization eggs
5	8	56.84	16.44	34.21
	16	54.31	23.51	31.55
	24	58.54	36.01	29.10
	Control	48.56	26.34	30.78
10	8	100.00	34.70	3.62
	16	100.00	16.91	43.30
	24	100.00	33.13	5.67
	Control	12.78	11.58	12.80
15	8	100.00	82.29	23.05
	16	100.00	92.58	50.30
	24	100.00	88.08	80.08
	Control	47.27	70.54	70.22

Table 5.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, egg stages. Percent mean excess mortality of three egg stages on the day of hatch, postthermal shock exposure data. (See Table 2 for explanation of excess mortality calculations.) The delta T 5 C° values are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent excess mortality		
		0 h post- fertilization eggs ¹	12 h post- fertilization eggs	24 h post- fertilization eggs
5	8	21.80	2.70	14.07
	16	18.84	5.88	12.57
	24	20.25	16.76	8.15
10	8	87.22	23.12	0
	16	87.22	5.33	33.29
	24	87.22	21.55	0
15	8	52.73	11.75	0
	16	52.73	22.04	0
	24	52.73	17.54	9.86

¹At delta T's 10 and 15 C°, 100% mortality occurred in the test samples at 0 h postfertilization egg stage. The numbers are less than 100% due to fluctuations in control sample mortalities.

Table 6.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, larval stages. Percent mean mortality of three larval stages at termination of experiment. The delta T 5 C° values are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent mean mortality		
		Day of hatch larvae	Day 2 larvae	Day 4 larvae
5	8	14.02	35.00	89.12
	16	17.17	40.25	91.64
	24	27.44	42.12	93.39
	Control	5.50	35.84	62.50
10	8	51.67	66.14	73.43
	16	38.33	51.67	84.56
	24	71.40	45.99	74.39
	Control	45.35	43.68	50.44
15	8	13.33	49.80	96.48
	16	17.59	37.30	72.88
	24	10.00	67.41	89.74
	Control	6.93	18.89	93.33

Table 7.--Thermal exposure experiment on mahimahi, Coryphaena hippurus, larval stages. Percent mean excess mortality of three larval stages at termination of experiment. (See Table 2 for explanation of excess mortality calculations.) The delta T 5 C° value are means of three separate experiments.

Delta T (C°)	Exposure time (min)	Percent excess mortality		
		Day of hatch larvae	Day 2 larvae	Day 4 larvae
5	8	10.72	10.40	26.62
	16	14.97	12.65	29.14
	24	23.16	12.23	30.89
10	8	21.43	27.01	22.99
	16	6.43	20.87	34.12
	24	26.05	8.10	23.95
15	8	8.71	30.91	3.15
	16	12.97	18.41	2.22
	24	3.71	48.52	0.47

Table 8.--Thermal exposure experiment on mahimahi, *Coryphaena hippurus*, egg and larval stages. Three-way ANOVA and Duncan multiple range test summary. Results shown are from arcsin transformation data analysis which have been converted back to percent mean mortality and percent excess mortality. The results of the three-way ANOVA and Duncan multiple range test are given for the three experimental variables: delta T, developmental stage, and exposure. Duncan multiple range test: x = mean; D-G = Duncan grouping, means with the same letter are not significantly different. Three-way ANOVA analysis: PR > F values, >0.05 = NS; <0.05 = *; <0.01 = **; <0.001 = ***.

Variable	Mahimahi combined						Mahimahi eggs						Mahimahi larvae					
	Percent mean mortality			Percent excess mortality			Percent mean mortality			Percent excess mortality			Percent mean mortality			Percent excess mortality		
	D-G ANOVA			D-G ANOVA			D-G ANOVA			D-G ANOVA			D-G ANOVA			D-G ANOVA		
	x			x			x			x			x			x		
Delta T (C°)	5	17.82	C	13.64	B		5.42	C		7.13	C		36.06	B		19.94	A	
	10	32.07	B	***	34.42	A	15.22	B	***	46.63	B	***	53.66	A	*	21.60	A	NS
	15	37.58	A		37.27	A	34.76	A		57.64	A		40.48	B		14.89	A	
Developmental stage																		
	0 h	34.63	B	52.47	A		34.63	A		52.47	A							
	12 h	12.32	C	20.29	B		12.32	B	***	20.29	B	***						
	24 h	0.24	D	0.83	C		0.24	C		0.83	C							
Larvae				***														
	DOH	12.86	C	16.63	B		--	--		--	--		12.86	C		16.63	A	
	Day 2	36.68	B	17.55	B	***	--	--		--	--		36.68	B	***	17.55	A	NS
	Day 4	81.44	A	23.73	B		--	--		--	--		81.44	A		23.73	A	
Exposure																		
	8 min	25.86	A	20.29	A		13.01	A		23.07	B		42.30	A		17.49	A	
	16 min	26.48	A	***	22.58	A	14.36	A	NS	26.71	A	NS	40.92	A	**	18.59	A	NS
	24 min	31.80	A		23.65	A	15.33	A		25.45	A,B		50.42	A		21.85	A	
	Control	13.31	B	--	--	--	3.91	B		--	--		27.72	B		--	--	

Table 9.--Thermal exposure experiment on manini, Acanthurus triostegus, egg and larval stages. Percent mean mortality of two egg stages and one larval stage.

Delta T (C°)	Exposure time (min)	Percent mean mortality		
		0 h post- fertilization eggs	18 h post- fertilization eggs	Day 1 larvae
5	8	25.29	0.71	45.43
	16	16.90	1.75	71.12
	24	19.14	0	60.00
	Control	24.25	1.52	32.03
10	8	40.89	24.88	43.15
	16	41.56	19.99	45.70
	24	44.94	14.97	50.26
	Control	28.46	16.27	32.19
15	8	91.00	0	85.93
	16	63.07	0.71	88.65
	24	43.51	18.71	89.49
	Control	6.20	0.77	50.43

Table 10.--Thermal exposure experiment on manini, Acanthurus triostegus, egg and larval stages. Percent mean excess mortality of two egg stages and one larval stage. (See Table 2 for explanation of excess mortality calculations.)

Delta T (C°)	Exposure time (min)	Percent excess mortality		
		0 h post- fertilization eggs	18 h post- fertilization eggs	Day 1 larvae
5	8	2.29	0.20	13.40
	16	0	1.25	39.09
	24	1.44	0	27.97
10	8	12.42	8.61	10.96
	16	20.44	4.38	13.51
	24	16.48	1.08	18.07
15	8	84.80	0	35.50
	16	56.87	0.45	38.22
	24	37.31	17.93	39.06

Table 11.--Thermal exposure experiment on manini, Acanthurus triostegus, egg and larval stages. Three-way ANOVA and Duncan multiple range test summary. Results shown are from arcsin transformation data analysis which have been converted back to percent mean mortality and percent excess mortality. The results of the three-way ANOVA and Duncan multiple range test are given for the three experimental variables: delta T, developmental stage, and exposure. Duncan multiple range test: x = mean; D-G = Duncan grouping, means with the same letter are not significantly different. Three-way ANOVA analysis: PR > F values, ≥ 0.05 = NS; < 0.05 = *; ≤ 0.01 = **; ≤ 0.001 = ***.

Variable		Percent mean mortality			Percent excess mortality		
		x	D-G	ANOVA	x	D-G	ANOVA
Delta T (C°)	5	17.21	B		9.69	B	
	10	32.81	A	***	11.88	B	***
	15	33.40	A		37.39	A	
Developmental stage							
Egg	0 h	34.48	B		28.98	A	
	18 h	4.27	C	***	3.78	B	***
Larvae	Day 1	56.58	A		26.43	A	
Exposure	8 min	31.26	A		20.69	A	
	16 min	31.21	A	***	20.67	A	NS
	24 min	31.78	A		18.17	A	
	Control	16.55	B		--	--	

Table 12.--Thermal exposure experiment on the striped mullet, Mugil cephalus, juvenile stage. Percent mean mortality and percent excess mortality is given, first, separately for each of two experiments conducted, then as a result of the two experiments combined.

Delta T (C°)	Exposure time (min)	Percent mean mortality		Percent excess mortality		Experiment I and II combined	
		Experiment I	Experiment II	Experiment I	Experiment II	% Mean mortality	% Excess mortality
5	8	0	5.00	0	0	2.00	1.00
	16	0	0	0	0	0	0
	24	0	0	0	0	0	0
	Control	0	5.00	--	--	2.00	--
10	8	0	10.00	0	10.00	4.00	4.00
	16	0	0	0	0	0	0
	24	0	5.00	0	5.00	2.00	2.00
	Control	0	0	--	--	0	--
15	8	50.00	65.00	50.00	65.00	56.00	56.00
	16	76.67	90.00	76.67	90.00	82.00	82.00
	24	80.00	90.00	80.00	90.00	84.00	84.00
	Control	0	0	--	--	0	--

Table 13.--Thermal exposure experiment on the striped mullet, Mugil cephalus, juvenile stage. Three-way ANOVA and Duncan multiple range test summary. Results shown are from arcsin transformation data analysis which have been converted back to percent mean mortality and percent excess mortality. The results of the three-way ANOVA and Duncan multiple range test are given for the three experimental variables: delta T, developmental stage, and exposure. Duncan multiple range test: x = mean; D-G = Duncan grouping, means with the same letter are not significantly different. Three-way ANOVA analysis: PR > F values, ≥ 0.05 = NS; < 0.05 = *; < 0.01 = **; < 0.001 = ***.

Variable	Percent mean mortality				Percent excess mortality			
		x	D-G	ANOVA		x	D-G	ANOVA
Delta T (C°)	5	0.10	B			0.33	B	
	10	0.15	B	***		2.01	B	***
	15	43.92	A			79.81	A	
Exposure	8 min	9.61	A			21.65	A	
	16 min	10.47	A	***		35.97	A	NS
	24 min	12.23	A			35.36	A	
	Control	0.05	B			--	--	

Table 14.--Predation experiment on mahimahi, *Coryphaena hippurus*, larval stages. Percent mean larvae consumed by predator at 10 min, 1 h, and 2 h time intervals in predation experiment I (130-165 ml/min flow rate). DOH = day of hatch.

Delta T (C°)	Exposure time (min)	Recovery time, 0 or 1 h	Percent mean mortality					
			10 min		1 h		2 h (total)	
			DOH	Day 1	DOH	Day 1	DOH	Day 1
5	8	0	22.9	1.7	75.0	8.5	91.7	28.8
		1	24.5	1.8	69.8	7.1	79.2	14.3
	16	0	14.9	0	70.2	12.7	89.3	25.5
		1	15.7	0	47.1	5.5	74.5	18.5
	24	0	17.3	0	44.2	5.3	82.7	7.0
		1	16.4	0	38.2	3.7	65.5	11.1
	Control	0	9.3	0	61.1	3.5	77.8	8.8
		1	17.0	0	47.2	5.2	73.6	8.6
10	8	0	11.1	9.6	24.4	15.4	35.5	36.5
		1	3.8	7.7	15.1	9.6	22.6	11.5
	16	0	1.9	11.3	13.2	13.2	20.7	17.0
		1	4.2	1.8	10.6	3.6	21.3	7.3
	24	0	5.6	5.6	17.0	9.4	18.9	13.2
		1	5.8	5.6	11.5	9.4	23.1	13.2
	Control	0	0	3.5	3.6	5.2	9.1	7.0
		1	3.4	0	13.5	0	18.6	0
15	8	0	10.3	8.6	15.5	32.7	24.1	43.1
		1	8.8	3.4	8.8	23.7	14.0	42.4
	16	0	12.5	1.8	12.5	20.3	21.4	24.1
		1	3.7	1.7	5.5	1.7	11.1	8.5
	24	0	8.8	13.8	12.3	19.0	17.5	29.3
		1	3.6	5.3	7.1	5.3	10.7	16.1
	Control	0	1.7	3.4	6.8	3.4	8.5	5.1
		1	1.7	3.4	1.7	3.4	3.4	3.4

Table 15.--Predation experiment on mahimahi, *Coryphaena hippurus*, larval stages. Percent mean larvae consumed by predator at 10 min, 1 h, 2 h, and 3 h time intervals in predation experiment II (flow rate 80-120 ml/min). DOH = day of hatch.

Delta T (C°)	Exposure time (min)	Recovery time, 0 or 1 h	Percent mean mortality							
			10 min		1 h		2 h		3 h (total)	
			DOH	Day 1	DOH	Day 1	DOH	Day 1	DOH	Day 1
5	8	0	5.2	1.7	7.0	5.0	15.8	6.7	19.3	8.3
		1	1.7	0	3.4	0	5.2	0	5.2	0
	16	0	5.3	3.3	8.9	18.3	32.1	31.7	53.6	40.0
		1	1.7	3.4	5.1	5.1	11.9	10.2	18.6	13.5
	24	0	1.7	3.6	5.1	3.6	10.2	3.6	23.7	3.6
		1	3.4	0	10.2	3.4	11.9	3.4	22.0	3.4
	Control	0	1.8	3.3	1.8	3.3	1.8	3.3	7.1	3.3
		1	1.7	0	3.3	0	5.0	1.7	5.0	3.4
10	8	0	5.5	7.5	14.8	11.3	31.5	15.1	37.0	17.0
		1	10.5	0	12.3	1.7	14.0	1.7	22.8	3.4
	16	0	3.5	3.7	17.5	9.2	54.4	16.7	63.1	20.4
		1	5.4	0	10.9	3.7	12.7	18.5	21.8	22.2
	24	0	5.3	1.7	16.1	7.0	26.8	10.5	35.7	14.0
		1	0	1.8	3.8	1.8	7.5	3.4	11.3	12.5
	Control	0	0	5.2	1.7	6.9	8.5	10.3	23.7	12.1
		1	3.4	1.7	3.4	1.7	10.2	1.7	18.6	1.7
15	8	0	5.3	8.1	10.7	17.7	14.3	21.0	26.8	37.1
		1	1.8	0	1.8	0	1.8	0	3.7	0
	16	0	12.5	8.6	23.2	22.4	28.6	27.6	39.3	34.5
		1	5.5	3.3	11.1	4.9	11.1	13.1	18.5	27.9
	24	0	3.6	5.1	10.9	6.8	14.5	8.5	21.8	10.2
		1	1.8	5.1	3.6	10.2	5.3	11.9	10.7	16.9
	Control	0	1.7	1.7	1.7	5.2	1.7	6.9	5.0	6.9
		1	0	1.7	0	1.7	0	3.3	1.7	3.3

Table 16.--Predation experiment on mahimahi, Coryphaena hippurus, larval stages. Percent mean larvae consumed by predator (excess mean mortalities due to predator) for predation experiments I and II. Excess mortality for experiment II calculated from 2 h (time interval) data. (See Table 2 for explanation of excess mortality calculations.) DOH = day of hatch.

Predation experiment series	Exposure time (min)	Recovery time, 0 or 1 h	Percent excess mortality					
			Delta T 5		Delta T 10		Delta T 15	
			DOH	Day 1	DOH	Day 1	DOH	Day 1
I	8	0	14.01	23.46	26.92	30.46	15.62	38.43
		1	8.14	8.24	5.35	11.34	10.47	37.70
	16	0	12.04	16.23	11.67	9.85	13.11	19.47
		1	3.12	7.11	4.44	7.41	7.60	5.37
	24	0	7.12	2.8	9.78	6.50	9.21	23.61
		1	1.66	5.86	4.43	13.07	7.04	11.34
II	8	0	14.03	4.37	25.05	6.70	12.59	13.91
		1	2.04	0	5.29	1.20	1.67	0
	16	0	30.12	28.34	45.88	6.77	26.69	20.61
		1	7.09	8.33	3.99	17.19	11.11	6.9
	24	0	10.26	0.97	18.89	3.54	12.57	4.38
		1	7.00	2.35	1.90	2.49	5.77	6.01

Table 17.--Predation experiment on mahimahi, *Coryphaena hippurus*, larval stages. Three-way ANOVA and Duncan multiple range test summary. Results shown are from arcsin transformation data analysis which have been converted back to percent mean mortality and percent excess mortality. The results of the three-way ANOVA and Duncan multiple range test are given for the three experimental variables: delta T, developmental stage, and exposure. Duncan multiple range test: x = mean; D-G = Duncan grouping, means with the same letter are not significantly different. Three-way ANOVA analysis: PR > F values, $>0.05 = \text{NS}$; $<0.05 = *$; $<0.01 = **$; $<0.001 = ***$. DOH = day of hatch; NS = not significant.

Variable	1 h recovery period					No recovery period				
	% mean mortality			% excess mortality		% mean mortality			% excess mortality	
	x	D-G	ANOVA	x	D-G	ANOVA	x	D-G	ANOVA	x
Mahimahi predation experiment I										
Delta T (C°)	5	10.93	B	5.70	B		41.56	A		12.64
	10	11.75	B	7.69	B	**	18.17	B	***	16.03
	15	34.33	A	13.41	A		18.07	B		20.23
Developmental stage	DOH	29.54	A	5.81	B		35.58	A		13.33
	Day 1	9.14	B	12.06	A	**	16.12	B	***	19.27
										A
Exposure	8 min	25.84	A	13.71	A		39.55	A		25.15
	16 min	16.49	B	5.85	B	**	29.30	A,B	***	13.83
	24 min	20.62	B,A	7.25	B		23.08	B		9.81
	Control	9.95	C	--	--		11.88	C		--
Mahimahi predation experiment II										
Delta T (C°)	5	3.93	A	4.47	A		8.45	B		14.97
	10	6.02	A	5.36	A	NS	18.27	A	*	18.24
	15	2.84	A	5.26	A		12.27	A,B		14.97
Developmental stage	DOH	5.49	A	5.11	A		15.44	A		22.11
	Day 1	3.03	A	4.96	A	NS	10.22	A	NS	10.12
										A
Exposure	8 min	1.37	C	1.70	B		14.85	B		12.94
	16 min	11.83	A	9.13	A	***	29.79	A	***	26.87
	24 min	5.32	B	4.20	B		9.10	B		8.49
	Control	1.52	C	--	--		3.46	C		--

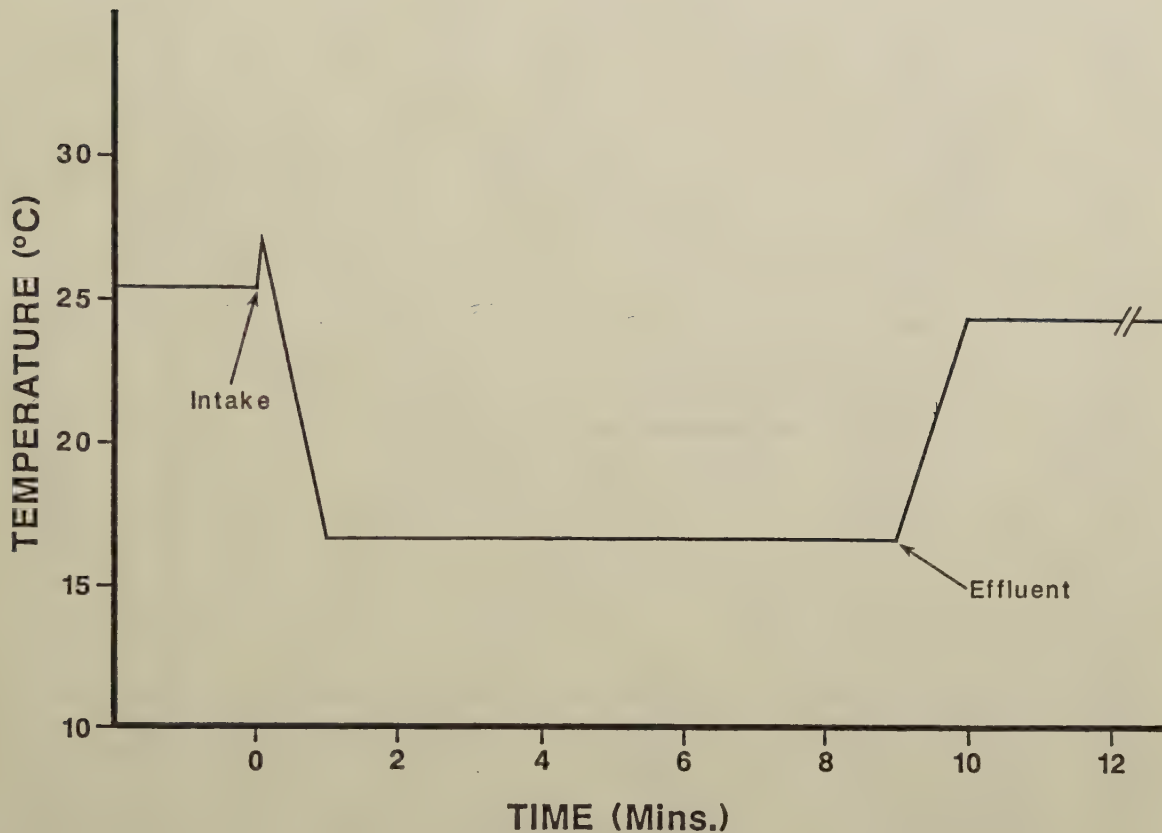


Figure 1.--Time-temperature history for a water parcel or organism entrained into the warmwater intake of the proposed Kahe Point facility (OTC 1983). At time zero, the water enters the plant, mixes with the heated effluent from the nearby oil-fired plant, decreases in temperature as it proceeds through the heat exchangers and then mixes with the cold water. The mixed effluent takes some 8 min to reach the terminus of the pipe, where it gradually increases to the ambient temperature at the release depth (near 100 m).

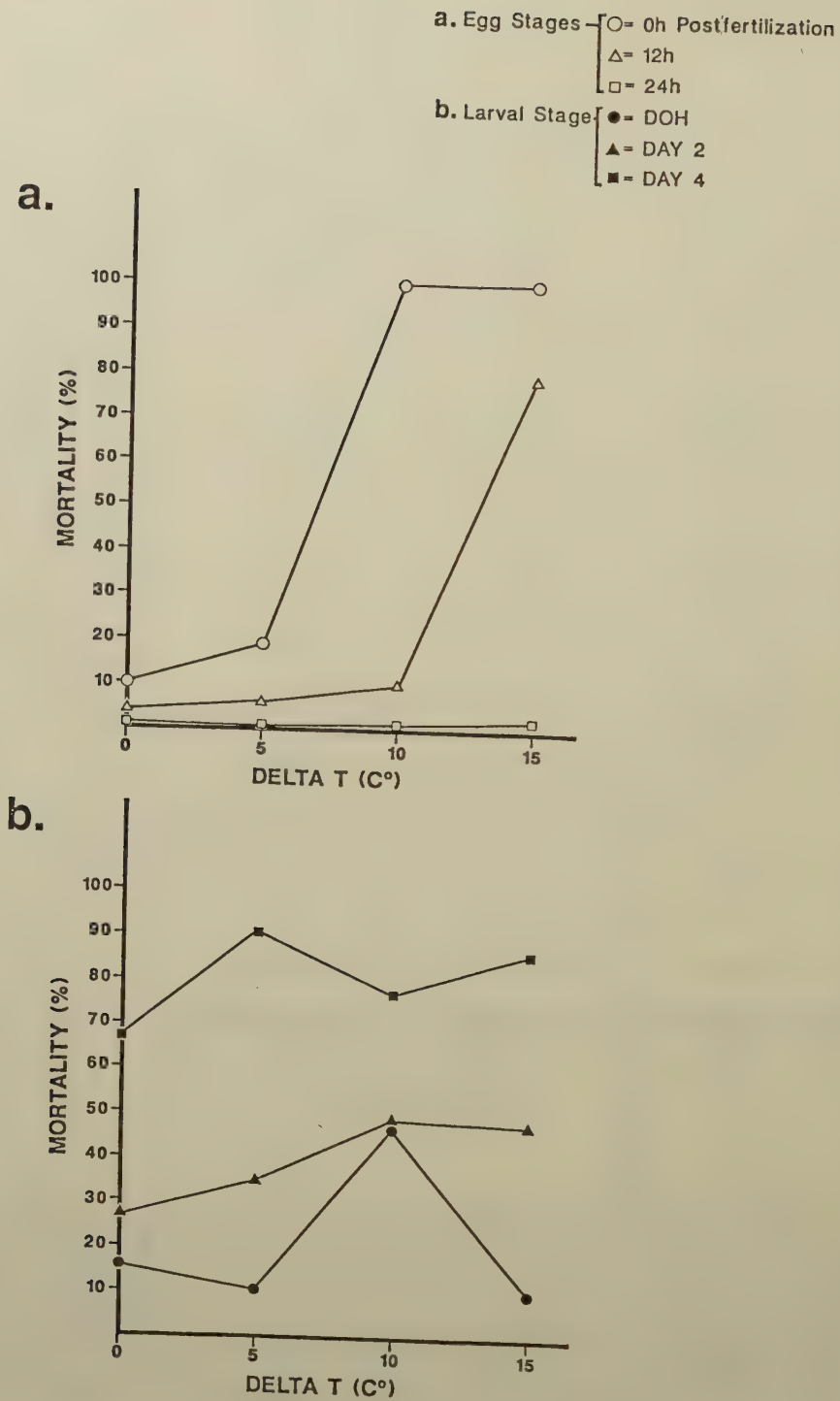


Figure 2.--Results of thermal exposure experiments on egg (A) and larval (B) stages of mahimahi, *Coryphaena hippurus*. Each data point is the mean of three replicates with the exception of the delta T 5 C° and the controls; the former is based upon three experiments and the latter the mean of controls run each day. Actual data points were arcsin transformed before taking means and means were transformed back to percent mean mortalities. Open circles, 0 h postfertilization; open triangles, 12 h postfertilization; open squares, 24 h postfertilization; solid circles, day of hatch larvae; triangles, day 2 larvae; squares, day 4 larvae.

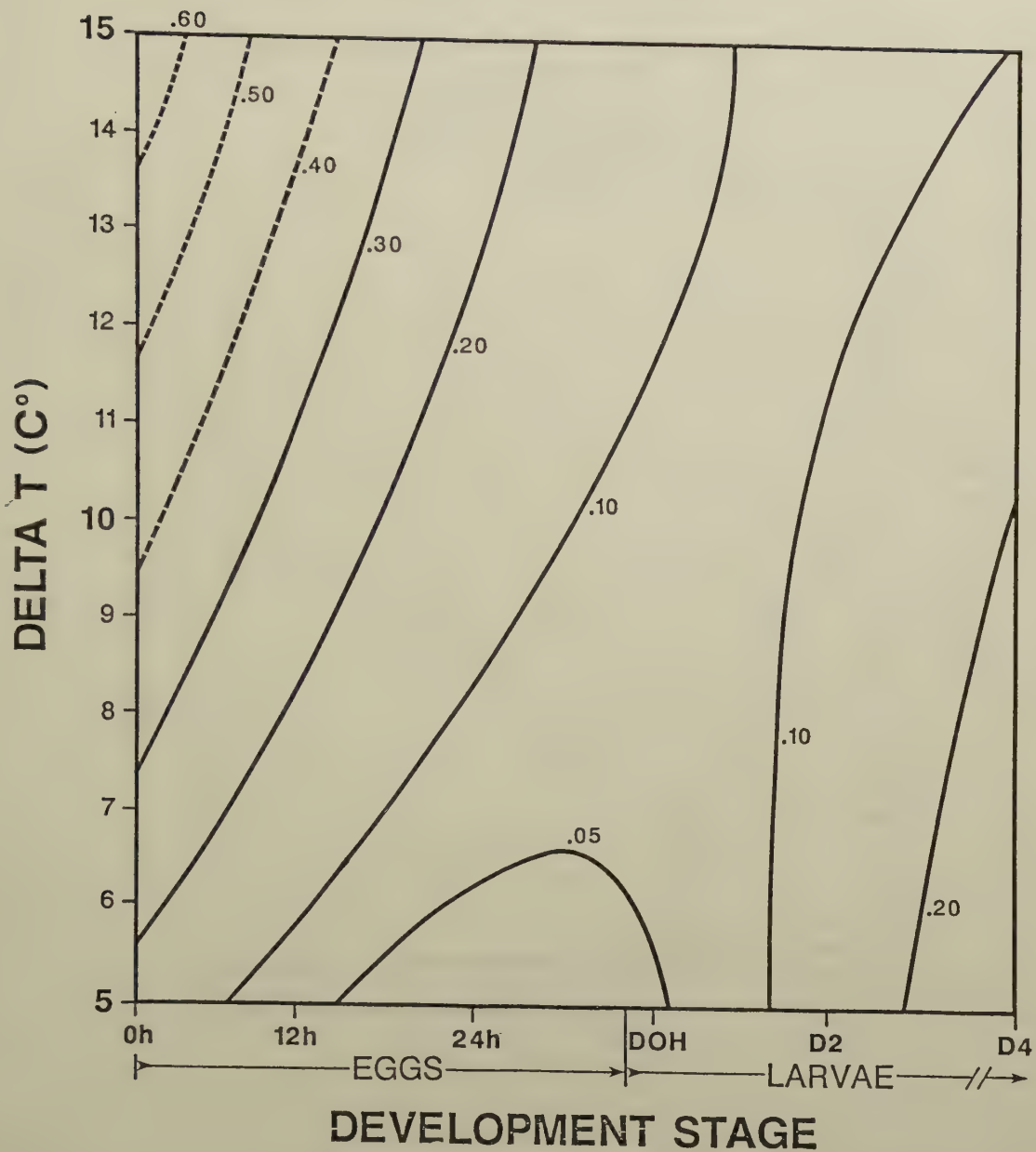


Figure 3.--Response surface model of excess mortality of egg and larval stages of mahimahi, *Coryphaena hippurus*. Data points were arcsin transformed before analysis and data transformed back to percent mean mortalities for presentation.

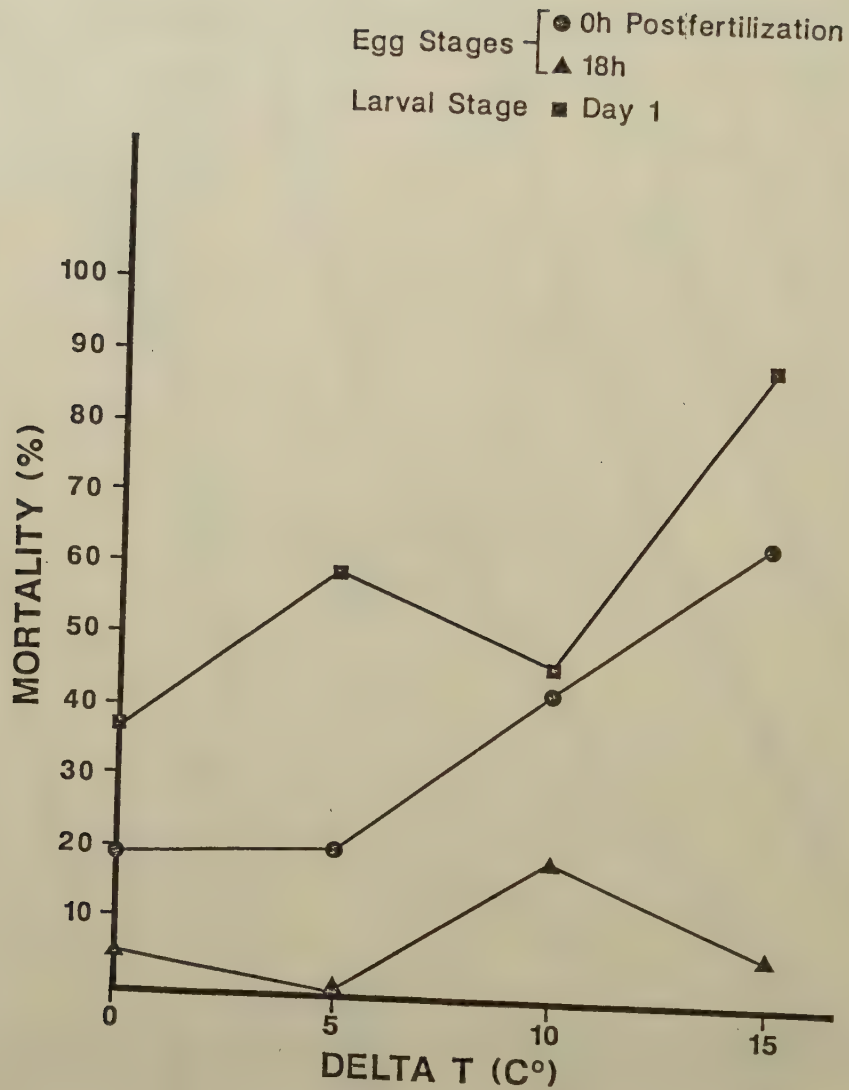


Figure 4.--Results of thermal exposure experiments on egg and larval stages of manini, Acanthurus triostegus. Actual data points were arcsin transformed before taking means and means were transformed back to percent mean mortalities. Circles, 0 h postfertilization; triangles, 18 h postfertilization; squares, day 1 larvae.

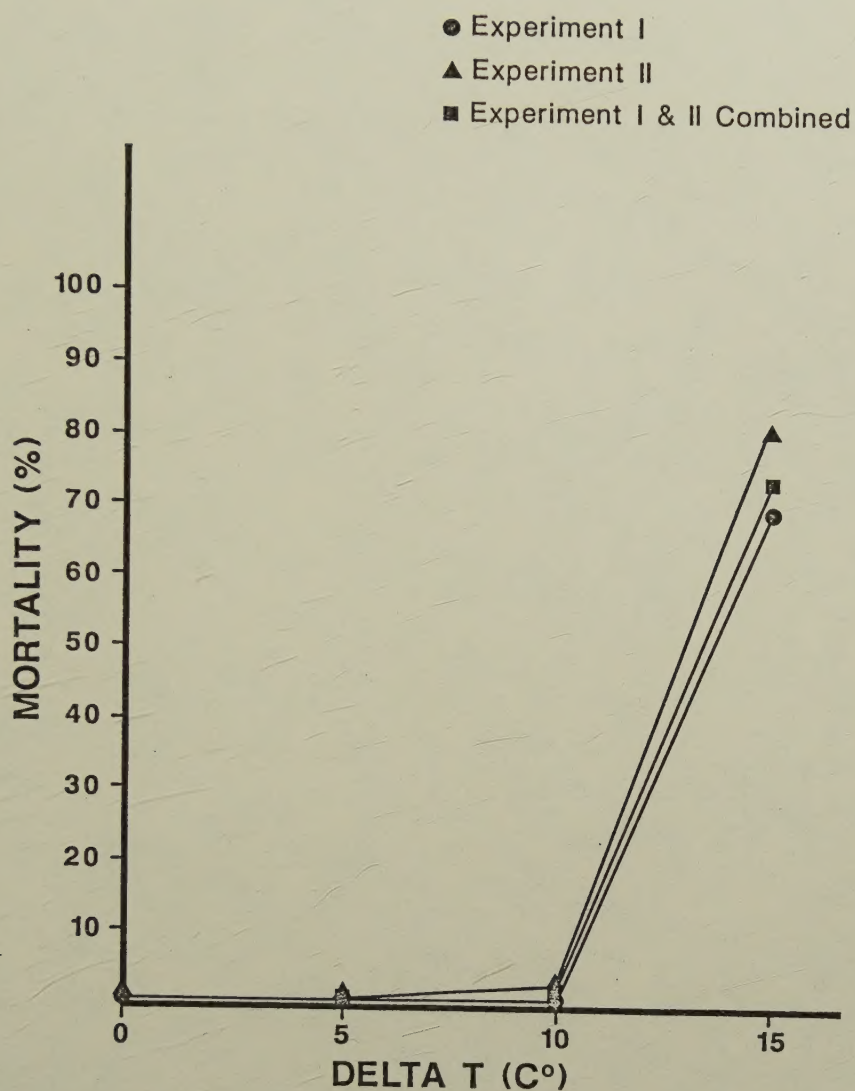


Figure 5.--Results of thermal exposure experiments on juvenile striped mullet, Mugil cephalus. Actual data points were arcsin transformed before taking means and means were transformed back to percent mean mortalities. Each data point is the mean of five replicates.

